

### ***Amendments to the Specification***

Please amend the pending paragraphs as follows

[0017] Next, explanation will be given of a method for concentration of nucleic acids. In this method, electrophoresis is performed twice so as to surely concentrate nucleic acids. Excessive ions in the sample are removed by the first electrophoresis, and the nucleic acids in the sample are concentrated by the second electrophoresis. Firstly, ~~[[1%]]~~ 100μL of 1% Triton (registered trademark) X-100, as the nonionic surfactant, is added to the sample containing the nucleic acids, and mixed, and then heated at 96°C for 10 minutes. Then, 100μL of 0.2% DPC, as the cationic surfactant, is added to the sample. Alternatively, both the nonionic surfactant and the cationic surfactant may be simultaneously added before the heat treatment. Even if the nucleic acids exist in procaryotic cells of colon bacillus or the like, cell walls are destroyed by the surfactants which are added to the sample for pretreatment. Accordingly, culture solution of colon bacillus or the like can be used as the sample, thereby facilitating the operation of pretreatment of the sample. The pretreatment is performed as the above, and direct voltage of 100V is applied so as to perform electrophoresis for 10 minutes, thereby removing the excessive ions from the sample. Subsequently, direct voltage of 125 to 150V is applied so as to perform electrophoresis for 120 minutes, thereby recovering the nucleic acids at the side of positive electrode.

[0047] Explanation will be given of an embodiment of the present invention. Firstly, an electrophoresis tank for electrophoresis will be described. Fig. 4 illustrates a first electrophoresis tank 21. An electrophoresis tank 21 is divided into a negative electrode side tank 22 and a positive electrode side tank 23 by partitions 24 and 25. The

partitions 24 and 25 are disposed at a center of the electrophoresis tank 21, and a sampling unit 26 is attached to the partitions 24 and 25. One end of the sampling unit 26 is projected into the negative electrode side tank 22, and the other end thereof is projected into the positive electrode side tank 23. A positive electrode side portion of the sampling unit 26 is filled with gel. A negative electrode side portion of the sampling unit 26 is filled with gel, and is provided in its side surface with an injection hole through which a sample is injected into the sample unit. At the time of electrophoresis, the injection hole is shut with a plug. A negative electrode is inserted into the negative electrode side tank 22 and a positive electrode is inserted into the positive electrode side tank 23 so as to apply voltage to the electrophoresis tank 21.

[0050] After removing excessive ions from the first electrophoresis tank 21, the connection part 33 and the filter part 34 are connected to the sampling unit 26. An [[O-ring]] O-ring is interposed in each junction so as to prevent leak of the solution. A solution prepared by mixing 100% ethanol and 1x TAE with the mixture ratio of 6:4 is supplied into the connection part 33, and TE-1 (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) is supplied into the ~~sampling unit 26~~ the filter part 34.

[0053] Explanation will be given of the connection part 33. Fig. 10 is a perspective view of the connection part, and Fig. 11 is a sectional side view of the connection part. Similar to the sampling unit 26, a centrifugal filter unit of Millipore is processed so that that an ultrafiltration membrane is removed therefrom and a hole having a 5mm diameter is opened therein, thereby constructing the connection part 33. The connection part 33 comprises a cylinder body 41 and a base 43. The cylinder body 41 is connected to the base 43. The base 43 is formed to be a stepped column, and a

vertical hole 44 penetrates the base 43. Gel 48 having thickness of several mm is disposed in the cylinder body 41. In the cylinder body 41, the gel 48 is disposed on an upper surface of the base 43 so as to prevent liquid from flowing from/to the ~~sampling unit 26.~~ filter part 34.

[0056] The connection part 33 is stood so as to turn the opening of the cylinder body 41 upward, and 1% agarose gel (SeaKem Gold agarose: bought from TaKaRa) is supplied into the cylinder body 41 through the opening so as to make the gel have several mm thickness, and then, the gel is hardened. Similar to the connection part 33, the sampling unit 26 is stood so as to turn the opening of the cylinder body 41 upward, and 1% agarose gel (SeaKem Gold agarose: bought from TaKaRa) is supplied into the cylinder body 41 through its opening so as to make the gel have several mm thickness. After the gel is hardened, the sampling unit 26 is reversed and the gel is supplied into the opening of the cylinder body 41 from an injection hole 42. HU-6 (made by AR BROWN) is used as the electrophoresis tank, and MPSU-200 (made by AR BROWN) is used as a power source.

[0057] The prepared sample for the electrophoresis is supplied into the sampling unit 26 through the ~~injector~~ injection hole 42, and then the hole is plugged. The electrophoresis tank is divided into the negative electrode side part and the positive electrode side part by putty, and 0.5x TAE is supplied into both side parts in the tank. The sampling unit 26 is disposed to the putty so as to make the upper surface of the sampling unit 26 upper than the buffer solution. Subsequently, direct voltage of 100V is applied and the first electrophoresis is performed for 20 minutes.

[0059] Explanation will be given of an embodiment of operation for the second electrophoresis. A solution is prepared by mixing 100% ethanol and 1x TAE with the mixture ratio of 6:4, and is supplied into the connection part 33. TE-1 (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) is supplied into the ~~sampling unit 26~~ filter part 34.

[0062] Next, the nucleic acid solution is recovered at the filter part 34 and its absorbance is measured by UV spectrum so as to calculate the concentration of the recovered nucleic acids. Fig. 14 illustrates a UV spectrum of the recovered solution. The calculated concentration of the nucleic acids is  $32.3\text{ng}/\mu\text{L}$  ( $6.7 \times 10^6$  [[106]] copies/ $\mu\text{L}$ ). The concentration of the nucleic acids is calculated so that the absorbance at 260nm (A260) is multiplied by a coefficient peculiar to the property of the nucleic acids, multiplied by an optical path length of a cell (mm), and divided by 10.

[0066] According to a method of the present invention for concentration and purification of a nucleic acid using electrophoresis, cationic surfactant and nonionic surfactant are added to a sample containing a nucleic acid so as to adjust electric charge of an impurity in the sample, and then the sample is placed in an electric field for electrophoresis so as to concentrate and purify the nucleic acid. Accordingly, the adsorption of the cationic surfactant can be adjusted by adjusting a ratio between the cationic surfactant and the nonionic surfactant, thereby easily adjusting migration of the electrophoresed impurity. ~~The nonionic surfactant adsorbs the nucleic acid so as to prevent the cationic surfactant from adsorbing the nucleic acid.~~

[0067] The electric charge of substance other than the nucleic acid is adjusted by the adsorption thereof the cationic surfactant, and the adsorption is adjusted by the added amount of the nonionic surfactant. Accordingly, the adsorption ratio of the impurity to

the cationic surfactant and the ratio of the nonionic surfactant can be operated easily.

Furthermore, the electric charge of the impurity can be adjusted by easy operation. ~~The nonionic surfactant adsorbs to the nucleic acid so as to prevent the cationic surfactant from adsorbing the nucleic acid.~~

[0072] The concentration unit ~~[[1]]~~ 101 in a t-DNA detector separates and concentrates target nucleic acids. The concentration unit 101 comprises an injection chamber 102, a separation chamber 108 formed of a separation medium, and a sampling chamber 103. A sample containing the target nucleic acids is introduced into the injection chamber 102 through a nozzle 109, and voltage is impressed on the injection chamber 102 and the sampling chamber 103 so as to make the nucleic acids migrate into the sampling chamber 103. The target nucleic acids having passed through the separation chamber 108 elute into the sampling chamber 103, and are sampled from the sampling chamber 103 through a nozzle 110. A biological sample, such as blood, urine, sputum or the like, a beverage, or a food can be used as the sample to be injected into the injection chamber 102. In addition, genome or plasmid can be injected.

[0075] Next, explanation will be given of concentration processes of the target nucleic acids by the concentration unit according to Fig. 18. Fig. 18 is a diagram of the concentration processes of the target nucleic acids. Firstly, Fig. 18 (a) shows the state that the sample is introduced into the injection chamber 102. For explaining plainly, herein, it is assumed that the sample contains target nucleic acids 112, nucleic acids 111 larger than the target nucleic acids 112 (bulkily or in molecular weight), and nucleic acids ~~[[112]]~~ 113 smaller than ~~[[the]]~~ target nucleic acids 112.

[0077] The preceding small nucleic acids 113 reach the sampling chamber 103 through the separation chamber 108. Then, the small nucleic acids 113 pass through the filter 104 and migrate to the electrode 106 in the sampling chamber 103. Subsequently, the target nucleic acids 112 reach the sampling chamber 103 through the separation chamber 108. Then, as shown in Fig. 18 (c), the filter 104 prevents the target nucleic acids 112 from further migrating toward the electrode 106. Since the filter 104 is shaped in a pyramid whose open bottom thereof faces to the separation chamber 108 and whose one open side surface is directed upward, the target nucleic acids 112 are concentrated in the part of the filter 104 toward the electrode [[106]] 105.

[0078] At this state, the voltage impression across the electrodes 105 and 106 is stopped and voltage is impressed across the electrodes 107 and 106 as shown in Fig. 18 (d). As a result, the electrode 106 still traps small impurities and nucleic acids 113, which are smaller than the target nucleic acids. When the voltage impression across the electrodes is stopped, the nucleic acids are liberated from the filter. In this way, during the liberation of the target nucleic acids 112 from the filter, and the small impurities and the small nucleic acids 113 are prevented from diffusing, thereby highly-accurately concentrating the target nucleic acids 112.

[0079] Then, the time for the target nucleic acids 112 to pass through the separation chamber 108 is calculated so as to determine the timing counted from the injection of the sample into the injection chamber [[2]] 102 for shift of the voltage impression from that across the electrodes 105 and 106 to that across the electrodes 107 and 106, thereby automatically performing separation and concentration of the target nucleic acids 112.

[0080] Any gel for electrophoresis, such as agarose gel, can be used as the separation chamber 108. A separation medium used as a column filler can also be used. For example, a carrier for gel filtration, such as Sephadex (registered trademark) (Pharmacia), can be used. It also is considerable that gel for electrophoresis and a column filler are combined at the separation part [[8]] 108 and adjusted so that the target nucleic acids flow out firstly.

[0082] Films 117a and 117b are stuck on an upper surface of the concentration unit 116, and the electrodes 105 and 106 are exposed on side surfaces of the concentration unit 116. The electrode [[7]] 107 is exposed on a bottom surface thereof. The electrode 105 is connected to the injection chamber, and the electrodes 106 and 107 are connected to the sampling chamber. The concentration unit 116 holds the separation chamber 108 therein, and the injection chamber and the sampling chamber are filled with buffer for electrophoresis. The sampling chamber is separated by the filter.

Please amend the abstract as follows:

In a method for concentration and purification method of nucleic acids using electrophoresis, cationic surfactant is added to a sample containing nucleic acids so as to adjust electric charge of impurities in the sample, and then the sample is placed in an electric field for electrophoresis so as to concentrate and purify the nucleic acids. The cationic surfactant (4) adsorbs substance other than the nucleic acids so as to adjust the electric charge of the substance, and the adsorption of the cationic surfactant (4) is adjusted by the added amount of nonionic surfactant (3). Alternatively, nucleic acids are

contacted with a separation medium (108), and then recovered by a filter (104) whose  
~~cross-sectional area is decreased in the direction of migration.~~